REMARKS

A Supplemental Information Disclosure Statement (sIDS) accompanies this response. A check for $705 for the requisite fee for a three-month extension of time ($525) and the fee for filing a supplemental IDS is enclosed. The Supplement Information Disclosure Statement has been filed under separate cover on the same day herewith.

Any fees that may be due in connection with the filing of this paper or with this application may be charged to Deposit Account No. 06-1050. If a Petition for extension of time is needed, this paper is to be considered such Petition.

Claims 1, 10-13, 20, 34-36, 40-46, 48-55, 108, 109, 113-116, 118-120 and 122-126 are pending. Claim 2, 3 and 19 are cancelled without prejudice or disclaimer. Claims 43-46, 48-55, 115, 116, 118-120 and 122-126 are withdrawn from consideration as directed to non-elected subject matter but are retained for possible rejoinder. Claim 1 is amended to include limitations of claim 19, which is cancelled, and also to emphasize that it is directed to a single chain polypeptide the consists only of the protease domain of an MTSP polypeptide. No new matter is added.

I. OBJECTION TO CLAIMS 11-13 AND 34 AS DIRECTED TO NON-ELECTED SUBJECT MATTER

Claims 11-13 and 34 are objected to as being drawn to non-elected subject matter. Applicant respectfully disagrees.

Each of claims 11-13 and 34 read on the elected species and are retained pending a determination of the allowability of claim 1. Claim 1, which is generic to the species recited in each of claims 11-13 and 34, is a linking claim directed isolated protease domains of MTSP polypeptides. Each of claims 11-13 and 34 is directed to isolated protease domains of MTSP polypeptides and, hence, are linked, by claim 1. Upon allowance of claim 1 the non-elected subject matter in these claims, which is within the scope of claim 1, also will be allowable.

II. THE REJECTION OF CLAIMS UNDER 35 U.S.C. §112, SECOND PARAGRAPH

Claims 1-3, 5, 11-13, 19, 20, 34-36, 40-42, 113 and 114 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter that applicant regards as the invention. The bases set forth by the Examiner are discussed in turn below. This rejection respectfully is traversed.

Relevant Law

Claims are not read in a vacuum but instead are considered in light of the specification and the general understanding of the skilled artisan. *Rosemount Inc. v. Beckman Instruments,*

Inc., 727 F.2d 1540, 1547, 221 USPQ 1, 7 (Fed. Cir. 1984), *Caterpillar Tractor Co. v. Berco, S.P.A.*, 714 F.2d 1110, 1116, 219 USPQ 185, 188 (Fed. Cir. 1983). A claim is not indefinite when one skilled in the art would understand the language in the claims when read in light of the specification.

35 U.S.C. § 112, second paragraph requires only reasonable precision in delineating the bounds of the claimed invention. Claim language is satisfactory if it reasonably apprises those of skill in the art of the bounds of the claimed invention and is as precise as the subject matter permits. *Shatterproof Glass Corp. v. Libby-Owens Ford Col.*, 758 F.2d 613, 624, 225 USPQ 634, 641 (Fed. Cir.), cert. dismissed, 106 S.Ct. 340 (1985).

Analysis

Claims 1-3, 5 and 11-13 are rejected under 35 U.S.C. § 112, second paragraph as allegedly being indefinite, because the Examiner urges that the metes and bounds of the recitation "substantially purified single-chain polypeptide" in claim 1 is not clear because the Examiner alleges that the specification does not provide a clear definition for the phrase "substantially purified." The Office Action states that "it is not clear to the Examiner as to how much of a presence of these readily detectable impurities qualifies an enzyme to be 'substantially pure.'"

Applicant respectfully disagrees. The specification defines what is meant by a "substantially pure" as used in the claims. For example, page 46, lines 4-15 recites:

> As used herein, substantially pure means sufficiently homogeneous to appear free of readily detectable impurities as determined by standard methods of analysis, such as thin layer chromatography (TLC), gel electrophoresis and high performance liquid chromatography (HPLC), used by those of skill in the art to assess such purity, or <u>sufficiently pure such that further purification would not detectably alter the physical and chemical properties</u>, such as enzymatic and biological activities, of the substance. Methods for purification of the compounds to produce substantially chemically pure compounds are known to those of skill in the art. A substantially chemically pure compound may, however, be a mixture of stereoisomers or isomers. In such instances, further purification might increase the specific activity of the compound.

Thus, it respectfully is submitted that, when read in light of the specification, the skilled artisan would understand the meaning of the recitation "substantially purified" as recited in the claims, that "substantially pure" enzyme appears free of detectable impurities as determined by standard methods of analysis, such that <u>further purification would not detectably alter the physical and chemical properties, such as enzymatic and biological activities, of the substance</u>. The skilled artisan readily would be able to determine the metes

and bounds of claims 1, 5, 11, 12 and 13. Applicant respectfully submits that, because the skilled artisan would understand the language and scope of the claims when read in light of the specification, the claims are not indefinite.

III. REJECTION OF CLAIMS 1-3, 5, 9, 11, 19, 20, 34-36, 40-42, 113 AND 114 UNDER 35 U.S.C. §112, FIRST PARAGRAPH - POSSESSION

Claims 1-3, 5, 9, 11, 19, 20, 34-36, 40-42, 113 and 114 are rejected under 35 U.S.C. §112, first paragraph, as allegedly containing subject matter that was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventor, at the time the application was filed, had possession of the claimed subject matter. The Examiner states that the claims are directed to a genus of polypeptides that comprise a protease domain or catalytically active portion thereof of a type-II membrane-type serine protease (MTSP) from any source including any or all recombinants, variants and mutants of MTSP or MTSP1. The Examiner alleges that the claims thus are allegedly drawn to polypeptides having any structure and allegedly are thus a structurally diverse genus. The Examiner states that the description of solely structural features present in all members of the genus is not sufficient to be representative of the attributes and features of the entire genus. The Examiner alleges that there is insufficient written description because the specification allegedly teaches only four species, and the Examiner contends that the disclosure of four species is not enough to describe the whole genus, and alleges that there is no evidence on record of the relationship between the structure of the serine protease domains of SEQ ID NOs. 2, 4, 6 and 11 and the structure of any or all MTSP polypeptides or a catalytically active portion of an MTSP polypeptide. The rejection respectfully is traversed.

Relevant Law

The purpose behind the written description requirement is to ensure that the patent applicant had possession of the claimed subject mater at the time of filing of the application. The relevant law and a discussion of the Patent Office Guidelines are set forth in the previous responses of record in this application, which are incorporated by reference herein. Briefly, the Federal Circuit has discussed the application of the written description requirement of the first paragraph of 112 to claims in the field of biotechnology. *See University of California v. Eli Lilly and Co.*, 119 F.3d 1559, 43 U.S.P.Q.2d 1398, 1406 (Fed. Cir. 1997). The court explained that:

> In claims involving chemical materials, generic formulae usually indicate with specificity what the generic claims encompass. One skilled in the art can distinguish such a formula from others and can identify many of

> the species that the claims encompass. Accordingly, such a formula is normally an adequate description of the claimed genus . . . a generic statement such as "vertebrate insulin or "mammalian insulin without more, is not an adequate written description of the genus because it does not distinguish the claimed genus from others, except by function. It does not specifically define any of the genes that fall within its definition. It does not define any structural features commonly possessed by members of the genus that distinguish them from others. One skilled in the art therefore cannot, as one can do with a fully described genus, visualize or recognize the identity of the members of the genus. A definition by function, as we have previously indicated, does not suffice to define the genus because it is only an indication of what the gene does, rather than what it is.

The court also stated that "[a]written description of an invention involving a chemical genus, like a description of a chemical species, 'requires a precise definition, such as by structure, formula, [or]chemical name,' of the claimed subject matter sufficient to distinguish it from other materials." *Id.* at 1567, 43 U.S.P.Q.2d at 1405. Finally, the court addressed the manner by which a genus of might be described. "A description of a genus of may be achieved by means of a recitation of a representative number of defined by nucleotide sequence, falling within the scope of the genus or of a recitation of structural features common to the members of the genus, which features constitute a substantial portion of the genus." *Id.*

The Federal Circuit also has addressed the written description requirement in the context of biotechnology-related subject matter in *Enzo Biochem. Inc. v. Gen-Probe*, 296 F.3d 1316, 63 USPQ2d (BNA) 1609 (Fed. Cir. 2002). The *Enzo* court adopted the standard that:

> the written description requirement can be met by 'showing that an invention is complete by disclosure of sufficiently detailed, relevant identifying characteristics . . . complete or partial structure, other physical chemical properties, functional characteristics when coupled with a known or disclosed correlation between function and structure, or some combination of such characteristics.'

The court in *Enzo* adopted its standard from the Written Description Examination Guidelines. The Guidelines apply to proteins as well as nucleic acid molecules.

It is well-settled that the written description requirement of 35 U. S. C. §112, first paragraph, can be satisfied without express or explicit disclosure of a later-claimed invention. *See, In re Herschler*, 591 F.2d 693, 700-01, 200 USPQ 711, 717 (CCPA 1979):

> "The claimed subject matter need not be described *in haec verba* to satisfy the description requirement. It is not necessary that the application describe the claim limitations exactly, but only so clearly that one having ordinary skill in the pertinent art would recognize from the disclosure that appellants invented processes including those limitations." (citations omitted).

See also Purdue Pharma L. P. v. Faulding, Inc., 230 F.3d 1320, 56 USPQ2d 1481 (Fed. Cir. 2000). In order to satisfy the written description requirement, the disclosure as originally filed does not have to provide *in haec verba* support for the claimed subject matter at issue.

The Claims

Claim 1 is directed to a substantially purified **single-chain** polypeptide **consisting only** of a protease domain of a type-II membrane-type serine protease (MTSP) or a catalytically active fragment thereof as a single chain, where the MTSP protease domain or catalytically active fragment thereof has serine protease activity as a single chain and **a free Cys in the protease domain is replaced with another amino acid**. Claim 5, 9, 11, 19, 20, 34-36, 40-42, 113 and 114 ultimately depend from claim 1 and are directed to various embodiments thereof. Thus, the claims are directed to isolated single chain MTSP protease domains.

Analysis

In setting forth the rejection, the Examiner urges that the specification does not set forth what specific structural or physical features define the claimed polypeptides and argues that one skilled in the art could not predict the structure and function of the claimed polypeptides that comprise a protease domain or catalytically active portion thereof of any or all MTSP polypeptides. The Examiner alleges that the genus of claim 1 and its dependent claims are structurally diverse because it encompasses any polypeptide that comprises a catalytically active protease domains of any or all MTSP or all MTSP1 and has serine protease activity. The Examiner states that the claims are drawn to polypeptides having any structure and are thus drawn to a genus encompassing species having substantial variation (Office Action, page 8) and that description of solely structural features present in all members of the genus is not sufficient to be representative of the attributes and features of the entire genus (see Office Action, page 9). Applicant respectfully submits that this is not correct.

Claim 1 and claims depending from claim 1 recite a substantially purified single-chain polypeptide consisting only of a protease domain of a type-II membrane-type serine protease (MTSP) or a catalytically active fragment thereof as a single chain, where the MTSP protease domain or catalytically active fragment thereof has serine protease activity as a single chain and a free Cys in the protease domain is replaced with another amino acid. Thus, the instant claims are directed to isolated single chain protease domains of an MTSP polypeptide. The instant application demonstrates that the isolated single chain protease domain of an MTSP polypeptide is active, and teaches replacement of a free cysteine with another amino acid, such as serine, to reduce aggregation. As discussed below, those of skill in the art can

identify protease domains in MTSPs, and, in view of the instant specification, which provides at least 12 exemplary protease domains (and identifies all family members known at the time of filing and provides at least four new MTSP protease domains (MTSP3, MTSP4 (2 variants) and MTSP6)as well as protease domains of known MTSPs). In light of this, it respectfully submitted, ludicrous to allege that the specification does not evidence that Applicant appreciated its invention at the time of filing. The application clearly and unequivocally spells it out"

> Provided herein are isolated protease domains of the Transmembrane Serine Protease family, particularly the Type II Transmembrain *[sic]* Serine Protease (TTSP) family (also referred to herein as MTSPs) . . ..

The specification also describes the sequences and provides references for all of the known members and teaches that a free Cys in the protease domain can be substituted with another amino acid.

The application identifies the 17 members of the MTSP family and members thereof (see, *e.g.*, page 4, lines *et seq.*) known at the time of filing, and provides the sequences of full-length MTSP proteases and identifies the protease domains thereof. These include MTSP1 and matriptase (also referred to as TAGD-15), MTSP3, MTSP4 (two variants encoded by splice variants), MTSP6, corin, enteropeptidase, human airway trypsin-like protease (HAT), hepsin, TMPRS2 and TMPRSS4. Further, the specification sets forth specific structural and physical features that define the protease domain of serine proteases. For example, the specification teaches, for example at page 19, lines 3-24, that:

> Exemplary MTSP proteins, with the protease domains indicated, are illustrated in Figures 1-3. Smaller portions thereof that retain protease activity are contemplated. The protease domains vary in size and constitution, including insertions and deletions in surface loops. They retain conserved structure, including at least one of the active site triad, primary specificity pocket, oxyanion hole and/or other features of serine protease domains of proteases. Thus, for purposes herein, the protease domain is a portion of a MTSP, as defined herein, and is homologous to a domain of other MTSPs, such as corin, enteropeptidase, human airway trypsin-like protease (HAT), MTSP1, TMPRSS2, and TMPRSS4, which have been previously identified; it was not recognized, however, that an isolated single chain form of the protease domain could function proteolytically in *in vitro* assays. As with the larger class of enzymes of the chymotrypsin (S1) fold (see, e.g., Internet accessible MEROPS data base), the MTSPs protease domains share a high degree of amino acid sequence identity. The His, Asp and Ser residues necessary for activity are present in conserved motifs. The activation site, which results in the N-terminus of second chain in the two chain forms is has a conserved motif and readily can be identified (see, e.g., amino acids 801-806, SEQ ID No. 62, amino acids 406-410, SEQ ID No. 64; amino acids 186-190,

> SEQ ID No. 66; amino acids 161-166, SEQ ID No. 68; amino acids 255-259, SEQ ID No. 70; amino acids 190-194, SEQ ID No. 72).

Further, the specification teaches, *e.g.*, at page 49, lines 3-10, that

> The MTSPs are a family of transmembrane serine proteases that are found in mammals and also other species that share a number of common structural features including: a proteolytic extracellular C-terminal domain; a transmembrane domain, with a hydrophobic domain near the N-terminus; a short cytoplasmic domain; and a variable length stem region containing modular domains. The proteolytic domains share sequence homology including conserved his, asp, and ser residues necessary for catalytic activity that are present in conserved motifs.

The specification describes the MTSPs and also how to identify a protease domain (see, *e.g.*, page 8):

> The protease domains as provided herein are single-chain polypeptides, with an N-terminus (such as IV, VV, IL and II) generated at the cleavage site (generally have the consensus sequence R↓VVGG, R↓IVGG, R↓ILGG, R↓VGLL, R↓ILGG or a variation thereof; an N-terminus of R↓V or R↓I, where the arrow represents the cleavage point) when the zymogen is activated. *To identify a protein domain an RI should be identified, and then following amino acids compared to the above noted motif[s].* [emphasis added]

In addition, the specification provides exemplary assays in which catalytic activity of the polypeptides can be tested (for example, see Examples 3 and 4). The art of record and discussed previously and herein evidences that, with the information provided in the specification, the skilled artisan can recognize the protease domain of a serine protease by its requisite protease domain structure and conserved features. If necessary, one of skill in the art could test the polypeptides for catalytic activity using the assays provided or known to those of skill in art to order to identify those that possess catalytic activity.

Hence, as discussed in more detail below, it is unequivocal that the instant application sufficiently describes the claimed polypeptides to demonstrate possession of the claimed subject matter at the time of the effective filing date of each claim. As is discussed in more detail below, to satisfy the written description requirement, one need not provide an example of every species encompassed by a claim. It is sufficient to provide identifying characteristics, including structural and physical characteristics, functional characteristics coupled with known or disclosed correlation with structural characteristics to demonstrate that the applicant was in possession of the claimed subject matter. MPEP § 2163; *see University of California v. Eli Lilly*, 119 F. 3d 1559, 1568, 43 USPQ2d 1398, 1406 (Fed. Cir.

1997). Further, the standard is an objective one, based on what one of skill in the art would recognize in the disclosure. *In re Gosteli*, 872 F.2d at 1012.

1. Members of the MTSP family of serine proteases were well known at the time of filing

First, as discussed previously, the MTSPs are a well known and characterized family of the serine proteases. The serine proteases were among the first enzymes to be studied extensively (Perona & Craik, *Protein Science* 4: 337-360 (1995)). The specification provides the sequence for almost a dozen exemplary MTSPs. The standard for evaluating written description is objective, based on what one of skill in the art would recognize in the disclosure. *In re Gosteli*, 872 F.2d at 1012. Hence, evaluation of written description takes into account the knowledge of one of skill in the art with regard to the particular subject matter.

a. <u>Type-II MTSP polypeptides have conserved characteristic structural elements</u>

The claimed polypeptides are single chain polypeptides the protease domain of a type-II MTSP or a catalytically active portion thereof. The serine protease family of enzymes has been extensively studied and characterized, evidenced by the art made of record in Information Disclosure Statements and provided in previous responses. Hooper *et al.* teaches that many of the serine proteases are mosaic proteins that include multiple, structurally distinct domains necessary for regulating enzymatic activity (*Eur. J. Biochem.* 267: 6931-6937 (2000)). Lin *et al.* ((1999) *J. Biol. Chem. 274*:18231-36) and Yan *et al.* ((1999) *J. Biol. Chem. 274*:14926-35)) teach that serine proteases are a family of proteins that can be distinguished from many other types of proteins and enzymes because they have highly conserved structures. For example, as discussed in the instant specification, it is known in the art that a substrate specificity pocket in the protease domain and conserved cysteines that participate in disulfide bonding are identified as highly conserved features in serine proteases (see, *e.g.*, Figure 4 and page 18235 of Lin *et al.* and Figure 2 and page 18236 of Yan *et al.*).

Type-II membrane-type serine proteases are a class of serine proteases characterized by having an NH_2-terminal cytoplasmic tail and a COOH-terminal ectodomain, lacking an NH_2-terminal cleavable signal sequence, and having a signal/anchor domain that anchors the serine protease in the cell membrane (*e.g.*, see Parks & Lamb, *J. Biol. Chem.* 268: 19101-19109 (1993) and Parks & Lamb, *Cell* 64: 777-787 (1991)). Tsuji *et al.* teaches that membrane-type serine proteases, such as hepsin, include a hydrophobic sequence flanked by a sequence having a positive net charges on the NH_2-terminal side while the COOH-terminal flanking side contains no charge, which agrees with the consensus topological sequence for

the type-II membrane proteins (Tsuji *et al.*, *J Biol Chem* 266(25): 16948-16953 (1991)). The membrane-type serine proteases have the triad of residues His57, Asp102 and Ser195 at the active site (chymotrypsin numbering system), are in close proximity and serve as a functional interacting unit responsible for bond formation and cleavage during catalysis (Craik *et al.*, *Science* 237:909-913 (1987). The instant specification teaches that the protease domain includes as a common structural feature a conserved catalytic triad. The art of record evidences that this is a characteristic feature. For example, Lin *et al.* teaches that membrane-type serine proteases include an invariant catalytic triad, a characteristic disulfide pattern and a proteolytic activation site in an Arg-Val-Val-Gly-Gly motif similar to the characteristic RIVGG motif in other serine proteases. (Lin *et al.*, *J Biol Chem* 274(26): 18231-18236 (1999)). Kitamoto *et al.* teaches that the catalytic domain of membrane type-II serine has a characteristic disulfide bond pattern (Kitamoto *et al.*, *Proc Natl Acad Sci USA* 91: 7588-7592 (1994)). Thus, a type II membrane protein can be characterized as a serine protease that includes the conserved catalytic triad, lacks a cleavable signal sequence, includes a transmembrane anchoring domain, and has positively charged residues on the N-terminal side of a long stretch of hydrophobic amino acids and has a characteristic disulfide bond pattern (Walter *et al.*, *Annu. Rev. Cell Biol.* 2: 499-516 (1986)). The lack of a signal sequence, a characteristic disulfide bond pattern, a characteristic hydrophobic region and the presence of a signal/anchor domain is also seen in other membrane-type serine proteases, including hepsin (Leytus *et al.*, *Biochemistry* 27: 1067-1074 (1988)), enteropeptidase (Kitamoto *et al.*, *Proc. Natl. Acad. Sci. USA* 91: 7588-7592 (1994)), TMPRSS2 (Poloni-Giacobino *et al.*, *Genomics* 44: 309-320 (1997)), and human airway trypsin-like protease (Yamakoka *et al.*, *J. Biol. Chem.* 273: 11895-11901 (1998)). Accordingly, the specification and the prior art sets forth specific structural and physical features that define the protease domain of serine proteases.

Further as noted above, the instant specification provides at least 17 members of the family and teaches sequences of at least 12 members and describes how to identify a protease domain (see, *e.g.*, page 8):

> The protease domains as provided herein are single-chain polypeptides, with an N-terminus (such as IV, VV, IL and II) generated at the cleavage site (generally have the consensus sequence R↓VVGG, R↓IVGG, R↓ILGG, R↓VGLL, R↓ILGG or a variation thereof; an N-terminus of R↓V or R↓I, where the arrow represents the cleavage point) when the zymogen is activated. *To identify a protein domain an RI*

> *should be identified, and then following amino acids compared to the above noted motif[s].* [emphasis added]

The polypeptides as instantly claimed include additional conserved structural features, such as a single chain protease domain and a free Cys residue that is replaced with another amino acid. The claims recite that the claimed polypeptides also have functional features, stating that the isolated protease domains of members of the type II transmembrane protease (MTSP) family possess protease activity as a single-chain polypeptide. These features are present in all members of the genus and are among the defining and requisite properties thereof. The specification clearly describes these features and demonstrates possession thereof.

b. <u>Protease domains of Type-II MTSPs are provided and are known in the art</u>

As discussed above and previously, the specification described protease domains of type-II MTSPs and provided sequences of exemplars thereof. For example, the specification teaches, *e.g.*, at page 19, lines 3-24, that:

> Exemplary MTSP proteins, with the protease domains indicated, are illustrated in Figures 1-3. Smaller portions thereof that retain protease activity are contemplated. The protease domains vary in size and constitution, including insertions and deletions in surface loops. They retain conserved structure, including at least one of the active site triad, primary specificity pocket, oxyanion hole and/or other features of serine protease domains of proteases. Thus, for purposes herein, the protease domain is a portion of a MTSP, as defined herein, and is homologous to a domain of other MTSPs, such as corin, enteropeptidase, human airway trypsin-like protease (HAT), MTSP1, TMPRSS2, and TMPRSS4, which have been previously identified; it was not recognized, however, that an isolated single chain form of the protease domain could function proteolytically in *in vitro* assays. As with the larger class of enzymes of the chymotrypsin (S1) fold (see, e.g., Internet accessible MEROPS data base), the MTSPs protease domains share a high degree of amino acid sequence identity. The His, Asp and Ser residues necessary for activity are present in conserved motifs. The activation site, which results in the N-terminus of second chain in the two chain forms is has a conserved motif and readily can be identified (see, e.g., amino acids 801-806, SEQ ID No. 62, amino acids 406-410, SEQ ID No. 64; amino acids 186-190, SEQ ID No. 66; amino acids 161-166, SEQ ID No. 68; amino acids 255-259, SEQ ID No. 70; amino acids 190-194, SEQ ID No. 72).

The specification also directs those skilled in the art to exemplary art that describes common structural features shared by the transmembrane serine proteases (for example, see page 18, lines 1-15). **Furthermore, the Type II transmembrane serine proteases are a recognized genus of polypeptides.**

As early as 1991, the serine protease catalytic domains for many serine proteases were understood or under investigation. For example, Tsuji *et al.* states that hepsin is a cell

membrane-associate serine protease that contains a short hydrophobic amino acid sequence in the region near the amino terminus while its carboxy-terminal half is a typical serine protease module (Tsuji *et al.*, *J Biol Chem* 266(25): 16948-16953 (1991). The full length sequences as well as the loci of the protease domains of a variety of MTSP serine protease family members were known at the effective time of filing the priority application and before. For example, Yan *et al.* (*J. Biol. Chem.* 274: 14926-14938 (1999)) and Tomia *et al.* (*J. Biochem.* 124: 784-789 ((1998)) describe the serine protease corin. Kitamoto *et al.* (*Biochem.* 27: 4562-4568 ((1995)), Yahagi *et al.* (*Biochem. Biophys. Res. Commun.* 219: 806-812 (1996)), Kitamoto *et al.* (*Proc. Natl. Acad. Sci. U.S.A.* 91: 7588-7592 (1994)), and Matsushima *et al.* (*J. Biol. Chem.* 269: 19976-19982 (1994)) describe enteropeptidase. Yamaoka *et al.* (*J. Biol. Chem.* 273: 11894-11901 (1998)) describes human airway trypsin-like protease. Leytus *et al.* (*Biochem.* 27: 11895-11901 (1988)), Vu *et al.* (*J. Biol. Chem.* 272: 31315-31320 (1997)) and Farley *et al.* (*Biochem. Biophys. Acta* 1173: 350-352(1993)) describe hepsin. Paoloni-Giacobino *et al.* (*Genomics* 44: 309-320 (1997)) and Jacquinet *et al.* (*FEBS Lett.* 468: 93-100 (2000)) describe TMPRS2. Wallrapp *et al.* (*Cancer* 60: 2602-2606 (2000)) describes TMPRSS4. International PCT application No. WO 00/52044 (which claims priority to U.S. application Serial No. 09/261,416) describes TADG-12. Each of these references is incorporated by reference in the instant application.

Lin *et al.* ((1999) *J. Biol. Chem. 274*:18231-36) and Yan *et al.* ((1999) *J. Biol. Chem. 274*:14926-35)) teach that serine proteases are a family of proteins that can be distinguished from many other types of proteins and enzymes because they have highly conserved structures. For example, a cleavage site at the N-terminus of the protease domain, a substrate specificity pocket in the protease domain and conserved cysteines that participate in disulfide bonding were identified as highly conserved features in serine proteases (see, *e.g.*, Figure 4 and page 18235 of Lin *et al.* and Figure 2 and page 18236 of Yan *et al.*). Lin *et al.* also teaches that when MTSP1 is compared to serine proteases, such as trypsin, chymotrypsin, enteropeptidase, TMPRSSR, other conserved elements are apparent, and these include a conserved activation motif ((R/K)VIGG), residues Asp627, Gly-655 and Gly-665 in the substrate pocket, with Asp at the bottom of the substrate pocket for trypsin-like proteases, such as MTSP1, and eight conserved cysteines needed to form intramolecular disulfide bonds (Lin *et al. J Biol Chem* 274(26): 18231-18236 (1999). In addition, a correlation between retention of the catalytic triad and retention of serine protease activity was demonstrated and known in the art at the time of filing. For example, Craik *et al.* (*Science* 237: 909-913 (1987)), Sprang *et al.* (*Science*

237: 905-909 (1987)), Carter *et al.* (*Nature* 332: 564-568 (1988)) and Bachovchin *et al.* (*Proc. Natl Acad. Sci.* 78: 7323-7326 (1981)) teach that serine protease activity is retained in a serine protease by retaining the conserved structure of the catalytic triad. Lin *et al.* ((1999) *J. Biol. Chem. 274*:18231-36) teaches that serine proteases are synthesized a single-chain zymogens that are proteolytically activated to become active two-chain forms (*e.g.*, see page 18235, col. 2, first full paragraph).

Comparing MTSP1 with other serine proteases, such as human trypsin, human chymotrypsin, the catalytic chains of human enteropeptidase, human hepsin, human blood coagulation factor XI, and human plasminogen, and the serine protease domains of two transmembrane serine proteases, human TMPRSS2 and Drosophila Stubble-stubbloid gene (Sb-sbd) show homologies in several structural features, including the catalytic triads, a conserved activation motif (R/KVIGG) and conserved cysteines that form intramolecular disulfide bonds (*e.g.*, see Lin *et al.*, J Biol Chem 274(26): 18231-18236 (1999). It is known in the art that membrane type II serine protease are mosaic proteins that include multiple, structurally distinct domains including the transmembrane domain and the protease domain, and that the sequences of several serine proteases demonstrate common highly-conserved features, including the catalytic triad and their surrounding motifs, disulfide-bond forming cysteine residues and the binding pocket (*e.g.*, see Hooper *et al.*, *Eur J Biochem* 267: 6931-6937 (2000)).

In addition, a correlation between retention of the catalytic triad and retention of serine protease activity was demonstrated and known in the art at the time of filing. For example, Craik *et al.* ((1987) Science 237:909-13), Sprang *et al.* ((1987) Science 237:905-09), Carter *et al.* ((1988) *Nature 332:*564-68) and Bachovchin *et al.* ((1981) *Proc. Natl Acad. Sci. 78*: 7323-26)) teach that serine protease activity could be retained in a serine protease by retaining the conserved structure of the catalytic triad. Lin *et al.* ((1999) *J. Biol. Chem. 274*:18231-36) teaches that serine proteases are synthesized a single-chain zymogens that are proteolytically activated to become active two-chain forms (*e.g.*, see page 18235, col. 2, first full paragraph). The MTSPs are described in the specification as a known family of proteases.

Thus, the specification provides all that was known about the serine proteases and the catalytic domains thereof at the time of filing, including that there are common conserved elements among the protease domains of MTSPs, such as the active site triad, a primary specificity pocket, an oxyanion hole, a conserved activation motif, a hydrophobic domain near the N-terminus and conserved cysteines that form intramolecular disulfide bonds.

2. Methods of identifying serine protease domains were known in the art at the time of filing and taught in the specification

As discussed above and previously, the serine proteases, including MTSP1, human trypsin, human chymotrypsin, the catalytic chains of human enteropeptidase, human hepsin, human blood coagulation factor XI, and human plasminogen, and the serine protease domains of two transmembrane serine proteases, human TMPRSS2 and Drosophila Stubble-stubbloid gene (Sb-sbd) show homologies in several structural features, including the catalytic triads, a conserved activation motif (R/KVIGG) and conserved cysteines that form intramolecular disulfide bonds (*e.g.*, see Lin *et al.*, J Biol Chem 274(26): 18231-18236 (1999). The locus of the protease domain for these proteins was known. The specification teaches that the MTSPs share common and conserved features. For example, see page 49, lines 3-13, which discloses:

> The MTSPs are a family of transmembrane serine proteases that are found in mammals and also other species that share a number of common structural features including: a proteolytic extracellular C-terminal domain; a transmembrane domain, with a hydrophobic domain near the N-terminus; a short cytoplasmic domain; and a variable length stem region containing modular domains. The proteolytic domains share sequence homology including conserved his, asp, and ser residues necessary for catalytic activity that are present in conserved motifs.

Dawson *et al.* (U.S. Pat. No. 5,645,833) teaches that the serine protease domain can be recognized by its homology with other serine proteases (col. 6, lines 29-32). As noted above, the application provides the amino acid sequences of numerous exemplary MTSPs. The application identifies and provides heretofore unknown MTSPs and provides full-length proteases and also the isolated protease domains. For example, the application identifies and provides heretofore unknown MTSPs MTSP3, MTSP4 and MTSP6 and also the isolated protease domains thereof. The application notes and describes known MTSPs and identifies the protease domains thereof.

The specification also defines structural features and structure-function relationships that identify the claimed genus of polypeptides consisting of a protease domain or catalytically active portion thereof and having serine protease activity. Such description includes information regarding the tertiary structure. For example, the specification teaches the locus of the disulfide bonds, identifies the Cys residues that link the protease domain to the rest of the polypeptide, and teaches that the polypeptide includes at least one of the active site triad, primary specificity pocket and oxyanion hole. The specification states that the serine protease family of proteins shares a high degree of homology. Hence, other related proteins, such as MTSPs from other species, can be readily identified by its homology with

other serine proteases. The specification also teaches that the protease domain of a MTSP shares homology and structural features with the chymotrypsin/trypsin family protease domains. The previous response and the application establish that the application describes the MTSP family and describes identification and isolation of protease domains.

The specification provides methods for identification, production, isolation, synthesis and/or purification of MTSP protease domains. The specification states, for example, that MTSP3, MTSP4 and MTSP6 are isolated from any animal, particularly a mammal, and includes but are not limited to, humans, rodents, fowl, ruminants and other animals (see page 20, lines 21-23; page 21, lines 11-13; and page 21, lines 29-31, respectively). Alternative methods for obtaining the MTSP protein than by directly isolating the MTSP protein also are provided. These include synthesis using genomic DNA, chemically synthesizing the gene sequence from a known sequence and making cDNA to the mRNA that encodes the MTSP protein, for example, and inserting the isolated nucleic acids into an appropriate cloning vector (for example, see pages 67-79).

The specification teaches the sequence of at least a dozen members of the family, incorporates by reference articles describing other members and explicitly teaches how to identify a protease domain. If that not sufficient, the specification also provides exemplary assays in which catalytic activity of the polypeptides can be tested (for example, see Examples 3 and 4). If necessary, one of skill in the art could test the polypeptides for catalytic activity using the assays provided or known to those of skill in art or to review the sequences to determine which possess the requisite protease domain structure in order to identify those that possess catalytic activity.

3. One of skill in the art would recognize Applicant's possession of the claimed subject matter

To satisfy the written description requirement, the issue is not whether the specification literally lists all of the possible MTSP protease domains and variants thereof that fall within the scope of the claims, but whether one of skill in the art in view of the specification would recognize that the applicant had provided a genus of single-chain polypeptides with the recited protease domain structure given the disclosure of the instant application in light of that which was known in the art. The specification provides relevant identifying characteristics of the genus of serine protease domains, including conserved structural and functional characteristics of an MTSP protease domain or catalytically active portion thereof, provides a number of exemplary protease domains, and the specification also directs those skilled in the art to

exemplary art that describes common structural and functional features shared by the protease domain of transmembrane serine proteases.

Therefore, the combination of the disclosure of the specific chemical structures of at least a dozen species within the scope of the claims as well as teachings in the specification (and knowledge of those of skill in the art) of how to identify a protease domain and also assays for testing for activity and the evidence that those of skill in the art are very familiar with the serine protease structure and function renders it clear that one of skill in the art would recognize that Applicant had possession of the claimed polypeptides at the time of the priority date of each claim. One of skill in the art would have recognized from reading the disclosure that Applicant had possession of this genus as well as numerous species thereof. This teaching and knowledge coupled with the ability to test for species within the scope of the claims with the assays provided for in the specification and known in the art demonstrates that Applicant sufficiently described and was in possession of the polypeptides as claimed, at the effective filing date(s) of the claims.

An adequate written description for a claimed genus only has to provide "relevant, identifying characteristics" of a representative number of species (MPEP §2163). The instant specification clearly meets this test. As noted, the specification provides at least a dozen examples of MTSPs and isolated protease domains (*e.g.*, see pages 9-10), including MTSP1, MTSP3, MSTP4 (2 splice variants) and MTSP6, incorporates publications describing all family members, and provides relevant structural and functional features that uniquely identify and specify the claimed genus of polypeptides. The specification teaches that those of skill in the art recognize common elements among MTSPs and the protease domains of MTSPs, and the specification teaches a number of conserved characteristics for the MTSPs and protease domains thereof, and the sequences and locus of the protease domains are known or can be determined as taught in the application. The specification teaches that members of the MTSP family are and were known, provides additional members, teaches how to identify and isolate protease domains as single chains and how to assess activity. One of skill in the art could, if needed, readily test any of the those polypeptides for catalytic activity. . The specification provides a large number of examples of MTSP protease domains, explicitly and implicitly and teaches that a contribution of Applicant is discovering that the protease domain has activity as a single chain; prior to Applicant's disclosure, it was believed that the protease activity required an activation cleavage to produce a two chain polypeptide. One of skill in the art, in

view of the instant disclosure, can prepare an active single chain protease domain from virtually any protease, including any MTSP.

The presently pending claims are directed to isolated single-chain protease domains, which the instant application teaches and demonstrates have activity as single chain polypeptides. The instant application provides the sequences of more than a dozen members of the family. Hence, the recitation in the claims that the polypeptides consist of a protease domain from an MTSP, are single-chain polypeptides having serine protease activity and a free Cys in the protease domain is replaced with another amino acid indicates "with specificity what the generic claims encompass. One skilled in the art can distinguish such a formula from others and can identify many of the species that the claims encompass." Therefore, the claims and application satisfy the standard set in *Eli Lilly* and the requirements of 35 U.S.C. §112, first paragraph.

In light of Applicant's disclosure, one of skill in the art would have recognized from reading the application that Applicant provided single-chain polypeptides with the recited protease domain structure that possess serine protease activity. Given the fact that numerous members of the MTSP family were known at the time of filing, the features of the protease domain of serine protease polypeptides are identified in the application and known in the art, coupled with the ability to test isolated single chain polypeptides for serine protease activity using assays provided in the application and known in the art, and to identify serine protease domains based on homology as known in the art and described in the specification, one of skill in the art would recognize that Applicant was in possession of the claimed subject matter at the effective filing date(s) of the claims.

IV. REJECTION OF CLAIMS 1-3, 5, 9, 19, 20, 34-36, 40-42, 113 AND 114 UNDER 35 U.S.C. §112, FIRST PARAGRAPH – Scope of Enablement

Claims 1-3, 5, 9, 19, 20, 34-36, 40-42, 113 and 114 are rejected under 35 U.S.C. § 112, first paragraph, because the specification allegedly fails to describe the claimed subject matter in such a way as to enable one skilled in the art to make and use the claimed subject matter commensurate in scope with these claims. The Examiner states that the specification is enabling for a polypeptide that includes amino acids 615-855 of SEQ ID NO:2, amino acids 205-437 of SEQ ID NO:4, amino acids of SEQ ID NO:6 and amino acids 217-443 of SEQ ID NO:112. The Examiner alleges that the specification does not reasonably provide enablement for a polypeptide that comprises any protease domain of any type II membrane type serine protease or catalytically portion thereof that include variants. The Examiner alleges that

predictability of which changes in a protein's amino acid structure can be tolerated requires a knowledge of and guidance with regard to the sequence as to which are tolerant to modification and which are conserved, and detailed knowledge of how the protein's structure relates to function. It is alleged that it would require undue experimentation for one of skill in the art to make such modified polypeptides with an expectation of success because the result of such modifications in unpredictable. It is further alleged that the claimed polypeptides encompass a large number of polypeptides and that the specification does not provide sufficient guidance on the nature of the changes that can be tolerated such that the proteins retain activity. In response to Applicant's arguments in the previous Response, evidencing the extensive knowledge in the art with respect to serine proteases, the instant Office Action argues that these arguments are not persuasive because the specification allegedly does not establish which specific amino acids in the protein's sequence can be modified such that the modified polypeptide continues to have proteolytic activity. The Examiner alleges that while the art may teach the general structure of MTSP and conserved amino acid sequences, protease domains, X-ray crystal structure and other attributes, such teachings "will not reduce the burden of undue experimentation on those of ordinary skill in the art." Therefore, the Office Action concludes, it would require undue experimentation to produce claimed polypeptides.

This rejection respectfully is traversed. As discussed below, above and previously, notwithstanding the disclosure of new proteases and individual protease claims, the instant application discloses and claims a generic invention: isolated **single-chain** protease domains (with a free Cys replaced with another amino acid) from any MTSP. The MTSP protein family is a well-known and well characterized family of proteins and its members were known in the art and/or disclosed in the application at the time of filing. In addition, the application provides some new members. The pending claims are directed to protease domains of a well-characterized family of proteins; there is no doubt that this family of proteins is well known and that those of skill in the art can identify members thereof. It is the instant application that teaches that the isolated single-chain protease domain possesses protease activity and that formation of a two-chain structure (by virtue of disulfide bonding with a Cys in the protease domain, which is free in the single chain form) is **not** needed. Thus the issue, is not identification of an MTSP, but identification of a protease domain in an MTSP. The application clearly teaches how to identify a protease domain and how to replace, the now free Cys that would have participated in forming a two chain structure. There are no issues regarding undue experimentation to isolate MTSPs.

Thus, the specification teaches identification, preparation and isolation of protease domains and those of skill in the art, in view of the application, readily can identify and isolate a protease domain from any MTSP. As discussed above, with respect to the written description rejection, the claims are directed to isolated single chain protease domains. The specification teaches that those of skill in the art can identify protease domains and also teaches how to identify protease domains. One of skill in the art, in light of the specification, could prepare an isolated single chain protease domain, as claimed, for any MTSP and replace the now-free Cys with another amino acid. Hence there is no reason to limit the claims to particular species of the family, when one of skill in the art, in light of the disclosure, can identify all members of the genus.

Relevant Law

The discussion of the relevant law from previous responses is incorporated herein.

Analysis

Application of the Factors Enumerated in *In re Wands*

It respectfully is submitted that analysis of enablement requires consideration of all of the "Wands Factors" and that focusing on one or two of the factors is a misapplication of the law. Applicant has discussed application of the "Wands Factors" in the previous responses, and such discussions are incorporated herein by reference. It would not require undue experimentation to isolate single-chain protease domains from any MTSP polypeptide. Further, it would not require undue experimentation to make modifications thereto. The Examiner admits that enzyme isolation techniques and recombinant and mutagenesis techniques are known in the art, and that it is routine in the art to screen for substitutions or modifications, including multiple substitutions and multiple modifications as encompassed by the instant claims (see Office Action, page 11). As discussed in detail below, and previously, a consideration of the factors enumerated in *In re Wands* demonstrates that the application teaches how to make and use the subject matter as claimed without undue experimentation.

Breadth of the Claims

Claim 1 is directed to an isolated substantially **purified single-chain polypeptide consisting only of a protease domain** of a type-II membrane-type serine protease (MTSP) or a catalytically active fragment thereof as a single chain, wherein the protease domain or catalytically active fragment thereof has serine protease activity as a single chain and **a free Cys** in the protease domain is replaced with another amino acid. Claims 20, 34-36, 40-42,

113 and 114 ultimately depend from claim 1 and recite additional features and specific family members.

Claim 20 recites that a free Cys in the protease domain is replaced. Claim 34 recites particular polypeptides within the scope of claim 1. Claims 35 and 36 are directed to conjugates including a polypeptide of claim 1 and a targeting agent linked to the protein directly or via a linker. Claims 40-42 are directed to a solid support including two or more polypeptides of claim 1 linked thereto either directly or via a linker. Claims 113 and 114 are directed to a solid support including two or more polypeptides of claim 12 linked thereto either directly or via a linker.

Hence the claims are directed to isolated protease domains of members of the MTSP family in which a fee Cys is replaced with another amino acid. The specification, as noted, describes all family members known and the time of filing and provides 4 new members of the family. Thus, the claims are of the same scope as the disclosure in the application.

Level of Skill

The level of skill in this art is recognized to be high (see, *e.g.*, *Ex parte Forman*, 230 USPQ 546 (Bd. Pat. App. & Int'f 1986)). The numerous articles and patents made of record in this application address a highly skilled audience and further evidence the high level of skill in this art.

Teachings of the Specification

As discussed above and previously, the specification teaches that MTSP polypeptides constitute a recognized well-known and well characterized family of serine proteases. For example, page 18, lines 1-23 recites:

> As used herein, "transmembrane serine protease (MTSP)" refers to a family of transmembrane serine proteases that share common structural features as described herein (see, also Hooper *et al.* (2001) J. Biol. Chem.276:857-860). Thus, reference, for example, to "MTSP" encompasses all proteins encoded by the MTSP gene family, including but are not limited to: MTSP1, MTSP3, MTSP4 and MTSP6, or an equivalent molecule obtained from any other source or that has been prepared synthetically or that exhibits the same activity. Other MTSPs include, but are not limited to, corin, enteropeptidase, human airway trypsin-like protease (HAT), MTSP1, TMPRSS2, and TMPRSS4. Sequences of encoding nucleic molecules and the encoded amino acid sequences of exemplary MTSPs and/or domains thereof are set forth in SEQ ID Nos. 1-12, 49, 50 and 61-72. The term also encompass MTSPs with conservative amino acid substitutions that do not substantially alter activity of each member, and also encompasses splice variants thereof. Suitable conservative substitutions of amino acids are known to those of skill in this art and may be made generally without altering the biological activity of the resulting molecule. Of particular interest are MTSPs of mammalian, including human, origin. Those of skill in this art recognize that, in

> general, single amino acid substitutions in non-essential regions of a polypeptide do not substantially alter biological activity (see, e.g., Watson *et al.* Molecular Biology of the Gene, 4th Edition, 1987, The Benjamin/Cummings Pub. Co., p.224).

The specification teaches that a protease domain from an MTSP polypeptide is active as a single-chain polypeptide. Additionally, smaller fragments of the protease domain also are active as single-chain polypeptides (page 18, line 24-page 19, line 2):

> As used herein, a "protease domain of an MTSP" refers to the protease domain of MTSP that is located within the extracellular domain of a MTSP and exhibits serine proteolytic activity. It includes at least the smallest fragment thereof that acts catalytically as a single chain form. Hence it is at least the minimal portion of the extracellular domain that exhibits proteolytic activity as assessed by standard assays in vitro assays. Those of skill in this art recognize that such protease domain is the portion of the protease that is structurally equivalent to the trypsin or chymotrypsin fold.

The specification further teaches that MTSP protease domains can vary in sequence but that these proteins retain a conserved structure as well as sequence identity to identified MTSP proteins exemplified in the application. For example, see page 19, lines 3-24, which recites:

> Exemplary MTSP proteins, with the protease domains indicated, are illustrated in Figures 1-3, Smaller portions thereof that retain protease activity are contemplated. The protease domains vary in size and constitution, including insertions and deletions in surface loops. They retain conserved structure, including at least one of the active site triad, primary specificity pocket, oxyanion hole and/or other features of serine protease domains of proteases. Thus, for purposes herein, the protease domain is a portion of a MTSP, as defined herein, and is homologous to a domain of other MTSPs, such as corin, enteropeptidase, human airway trypsin-like protease (HAT), MTSP1, TMPRSS2, and TMPRSS4, which have been previously identified; it was not recognized, however, that an isolated single chain form of the protease domain could function proteolytically in *in vitro* assays. As with the larger class of enzymes of the chymotrypsin (S1) fold (see, e.g., Internet accessible MEROPS data base), the MTSPs protease domains share a high degree of amino acid sequence identity. The His, Asp and Ser residues necessary for activity are present in conserved motifs. The activation site, which results in the N-terminus of second chain in the two chain forms is has a conserved motif and readily can be identified (see, e.g., amino acids 801-806, SEQ ID No. 62, amino acids 406-410, SEQ ID No. 64; amino acids 186-190, SEQ ID No. 66; amino acids 161-166, SEQ ID No. 68; amino acids 255-259, SEQ ID No. 70; amino acids 190-194, SEQ ID No. 72).

The application describes the protease domain of a number of MTSP family members including MTSP1, MTSP3, MTSP4 and MTSP6 as well as HAT, corin, enteropeptidase, TMPRSS4 and TMPRSS2, identifies all members known at the time, and provides the full-length sequence for at least a dozen members, and, as noted, identifies 4 new family members.

As discussed above, identification of the protease domain from an MTSP region merely requires identification of the activation cleavage site and as outlined in the specification, discussed above and known in the art. The locus of the protease domain in the known MTSP

family members is known, and the instant application provides protease domains from several other family members.

Furthermore, notwithstanding that the protease domain of all members of the family known at the time of filing, plus the 4 additional family members provided in the application, a comparison of sequence identity among family members (see, *e.g.*, Figure 4 of the application) reveals that the protease domains share conserved sequences, including the catalytic triad of His, Asp and Ser residues and their surrounding conserved motifs. Additionally, the specification demonstrates that MTSP protease domains can have a reasonable amount of sequence variation and yet retain serine protease activity. MTSP1, MTSP3, MTSP4 and MTSP6 protease domains share about 40% sequence identity with each other. The specification teaches that each of these protease domains is an example of an MTSP protease domain that has activity in the single chain form.

The specification also teaches additional modifications. For example, see page 26, lines 13-25, which recites:

> Hence smaller portions of the protease domains, particularly the single chain domains, thereof that retain protease activity are contemplated. Such smaller versions will generally be C-terminal truncated versions of the protease domains. The protease domains vary in size and constitution, including insertions and deletions in surface loops. Such domains exhibit conserved structure, including at least one structural feature, such as the active site triad, primary specificity pocket, oxyanion hole and/or other features of serine protease domains of proteases. Thus, for purposes herein, the protease domain is a single chain portion of an MTSP, as defined herein, but is homologous in its structural features and retention of sequence of similarity or homology the protease domain of chymotrypsin or trypsin. Most significantly, the polypeptide will exhibit proteolytic activity as a single chain.

The specification teaches that included in the conserved features of MTSP protease domain polypeptides is a catalytic triad as well as the activation cleavage site, which defines the terminus of the protease domain polypeptides when they are isolated as single chain polypeptides.

The specification explains that beyond such conserved features the polypeptides are tolerant of modification. The specification explains that such modifications can be effected using numerous methods known in the art. For example, at page77, line 17 through page 78, line 11, the specification states:

> A variety of modifications of the MTSP proteins and domains are contemplated herein. An MTSP-encoding nucleic acid molecule can be modified by any of numerous strategies known in the art (Sambrook *et al.*, 1990, Molecular Cloning, A Laboratory Manual, 2d ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York). The sequences can be cleaved at appropriate sites with

restriction endonuclease(s), followed by further enzymatic modification if desired, isolated, and ligated in vitro. In the production of the gene encoding a domain, derivative or analog of MTSP, care should be taken to ensure that the modified gene retains the original translational reading frame, uninterrupted by translational stop signals, in the gene region where the desired activity is encoded.

Additionally, the MTSP-encoding nucleic acid molecules can be mutated in vitro or in vivo, to create and/or destroy translation, initiation, and/or termination sequences, or to create variations in coding regions and/or form new restriction endonuclease sites or destroy pre-existing ones, to facilitate further in vitro modification. Also, as described herein muteins with primary sequence alterations, such as replacements of Cys residues and elimination of glycosylation sites are contemplated. Such mutations may be effected by any technique for mutagenesis known in the art, including, but not limited to, chemical mutagenesis and in vitro site-directed mutagenesis (Hutchinson *et al.*, J. Biol. Chem. 253:6551-6558 (1978)), use of TAB® linkers (Pharmacia). In one embodiment, for example, an MTSP protein or domain thereof is modified to include a fluorescent label. In other specific embodiments, the MTSP protein is modified to have a heterofunctional reagent, such heterofunctional reagents can be used to crosslink the members of the complex.

The specification exemplifies variation in MTSP sequences. For example the specification provides exemplary MTSP1, MTSP3, MTSP4 and MTSP6 sequences. The specification explains that MTSP1 and MTSP3 amino acid sequences have about 43% identity with each other (for example, see page 162, lines 1-2). The specification also discloses that MTSP1 and MTSP4 have about 37% amino acid sequence identity (for example, see page 167, lines 25-29). The specification also teaches that MTSP4 and MTSP6 share about 60% amino acid sequence identity (for example, see page 172, lines 4-9). The specification teaches that each of the protease domains of these MTSP family members is active as single chain that contains only the protease domain or a smaller catalytically active portion of the protease domain (see, for example at page 20, lines 1-6). Hence, the specification teaches that MTSP protease domains share about 40%-60% and greater sequence identity and are active as single chain polypeptides.

The specification teaches additional modifications of the MTSP polypeptides. For example, the specification explains that for each individual MTSP, the polypeptides can include about 60% amino acid sequence identity with the exemplified MTSP. Such modified polypeptides exhibit serine protease activity as single chain polypeptides. The specification provides exemplary modifications including conservative amino acid substitution (for example, see page 10, lines 3-13) and modifications of cysteine residues and/or of glycosylation sites (for example, see page 78, lines 1-7). The specification also discloses that non-natural amino acids can be introduced as a substitution or addition in the MTSP polypeptides (for example, see page 79, lines 10-21).

More significantly, the pending claims, are directed, not to full-length MTSPs, but isolated single-chain protease domains. One of skill in the art with an MTSP in hand, could readily identify the protease domain as claimed and replace a free Cys.

Knowledge of those of skill in the art

As discussed above, at the time of filing of the application and before, those of skill in the art were very familiar with serine proteases generally, and with the MTSP family in particular. The family was known as was the locus of the protease domain. What was absent was any understanding or recognition that an isolated single chain protease domain would have activity; hence, such was never isolated. In view of the instant application teaching that such protease domains have activity as single chains, the skilled artisan can readily isolate any protease domain as a single chain of an MTSP. Nothing more need be known regarding the requisites for activity.

Notwithstanding this, there was a large body of literature directed to serine proteases and there was general understanding of their structures and requisites for activity (see for example, Hooper *et al. J. Biol. Chem. 276*:857-860, Nienaber *et al.* (2000) *J. Biol. Chem.* 275:7239-48; Sommerhoff *et al.* (1999) *Proc. Natl. Acad. Sci. USA* 96:10984-91; Lu *et al.* (1999) J. Mol. Biol. 292:361-73; Xu *et al.* (2000) *J. Biol. Chem.* 275:378-385, Lin *et al.*(1999) *J. Biol. Chem. 274*: 18231-36 and Bryan (2000) *Biochem. Biophys. Acta 1543*:200-03). These references detail the existing crystal structures, structural comparisons and structural similarities of serine proteases.

This extensive knowledge also is evidenced, for example, in the application as filed and in the literature made of record in the submitted Information Disclosure Statements. As noted in the application, the Type II Serine Proteases family (TTSPs), also referred to as MTSPs, were known (for example, see pages 4-5). Serine proteases are a family that can be distinguished from many other types of proteins and enzymes because they have highly conserved structures (see *e.g.*, Lin *et al.* (1999) *J. Biol. Chem. 274*:18231-36 and Yan *et al.* (1999) *J. Biol. Chem. 274*:14926-35). Moreover, it was known at the time of filing that there is a known correlation between retention of the catalytic triad and retention of serine protease activity. Hence, available to one of skill in the art was the knowledge that serine protease activity could be retained in a serine protease by retaining the conserved structure of the catalytic triad (see for example, Carter *et al.* (1988) *Nature 332:*564-68, Sprang *et al.* (1987) Science 237:905-09, Craik *et al.* (1987) Science 237:909-13 and Bachovchin *et al.* (1981) *Proc. Natl Acad. Sci. 78*: 7323-26). In addition, other features were identified at the time of filing as highly conserved features in serine proteases including a cleavage site at the N-

terminus of the protease domain, a substrate specificity pocket in the protease domain and conserved cysteines that participate in disulfide bonding (see for example, Figure 4 and page 18235 of Lin *et al.* and Figure 2 and page 18236 of Yan *et al.*) Hence, the requisites for retention of serine protease activity are well-known and characterized and were available at the effective filing date of the claimed subject matter. Hence, a wide variety of structural information on serine proteases was well-known in the art.

Furthermore, the instant claims only require identification of the protease domain of an MTSP, and its isolation as a single chain polypeptide. A number of MTSPs were known and the locus of the protease domain identified. Those of skill in the art can readily identify the protease domain region in an MTSP, and, if necessary test it for the protease activity.

The methods and guidance for comparing amino acid sequences to generate and confirm sequences with sequence identity to an MTSP polypeptide sequence such as SEQ ID NOS: 2, 4, 6 and 12 was available in the art at the time of filing the instant application. As described in the instant specification, computer algorithms such as the "FAST A" program, using for example, the default parameters as in Pearson *et al.* (1988) Proc. Natl. Acad. Sci. USA 85:2444 were available. Other available programs include the GCG program package (Devereux, J., *et al.* , Nucleic Acids Research 12(I):387 (1984)), BLASTP, BLASTN, and FASTA (Atschul *et al.*, J Molec Biol 215:403 (1990); *Guide to Huge Computers*, Martin J. Bishop, ed., Academic Press, San Diego, 1994, and Carillo *et al.* (1988) SIAM J Applied Math 48:1073). In addition, methods for generating nucleotide and protein sequence variation were widely available in the art. Thus, one of skill in the art could use such programs with a serine protease sequence, for example, to align the sequence and identify the structural features of importance for retention of activity and use the methods for generating sequence variation to make the identified protein variants. The Examiner states that enzyme isolation techniques and recombinant and mutagenesis techniques are well known.

Methods for assaying protease activity including protease specificity, level of activity and response to inhibitors was well known in the art (see, for example, Lu *et al.* (1999) J. Mol. Biol. 292:361-73; Xu *et al.* (2000) *J. Biol. Chem.* 275:378-385). Methods for high throughput assays and detection were also widely available (See generally, *High Throughput Screening: The Discovery of Bioactive Substance*s (Devlin, Ed.) Marcel Dekker, 1997; Sittampalam *et al., Curr. Opin. Chem. Biol.*, 1:384-91 (1997); and Silverman *et al., Curr. Opin. Chem. Biol.*, 2:397-403 (1998)). Hence, the amount of knowledge of those of skill in

the art was extensive and the requisite structural and functional features required for protease activity was well known.

The Examiner states that the specific amino acid positions within a protein's sequence where amino acid modification can be made with a reasonable expectation of success in obtaining the desired activity are limited in any protein and the result of such modifications is unpredictable. Applicant respectfully disagrees in the case of the family of serine proteases. The application and the art made of record establish that serine proteases are well known in the art and the structural requirements for activity are known and that the instantly claimed polypeptides share sequence homology with the chymotrypsin/trypsin family for which tertiary structures are known. For example, it was known in the art that serine protease activity could be retained in a serine protease by retaining the conserved structure of the catalytic triad (see *e.g.*, Craik *et al.*, Science 237:909-13 (1987), Sprang *et al.*, Science 237:905-09 (1987), Carter *et al.*, *Nature 332:*564-68 (1988) and Bachovchin *et al. Proc. Natl Acad. Sci. 78*: 7323-26 (1981)). Other highly conserved features in serine proteases also were known to the skilled artisan. These include a cleavage site at the N-terminus of the protease domain, a substrate specificity pocket in the protease domain and conserved cysteines that participate in disulfide bonding (see for example, Figure 4 and page 18235 of Lin *et al.* and Figure 2 and page 18236 of Yan *et al.*). The specification also provides exemplary assays for testing catalytic activity of the polypeptides using routine experimental analysis techniques and also provides descriptions of how to assess percentage identity and teaches that these techniques were well known in the art. The specification also teaches conserved characteristics among serine proteases. Furthermore, the MTSPs are a known family of serine proteases, and the protease domain of any member can be readily identified.

The Examiner states that recombinant and mutagenesis techniques and enzyme isolation techniques are known and that it is routine to screen for multiple substitutions or multiple modifications as encompassed by the instant claims (see Office Action, page 11). Thus, routine techniques can be used to identify or synthesize modified MTSP serine protease domains. If needed, one of skill in the art can test polypeptides for catalytic activity by routine experimentation using the assays provided in the specification or known to those of skill in art. Furthermore, the instant claims are directed to single chain protease domains of MTPSs, which are known. Issues regarding modification and requisites therefor are irrelevant.

Working Examples

The application provides working examples that demonstrate each of the features of the claimed polypeptides. For instance, the Examples provide detailed guidance for identifying and isolating MTSP protease domains. Example 1 describes the cloning of and identification of MTSP3 based on its sequence similarity with MTSP1. Example 2 describes the identification and cloning of two MTSP4 polypeptides, MTSP4-S and MTSP4-L. Example 3 describes the identification and cloning of an MTSP6 polypeptide based on sequence similarity to MTSP4. In each case, an MTSP polypeptide sequence is identified that includes a protease domain with a cleavage site and a catalytic triad (see, e.g., Figure 4). As noted, for example, in Example 1, identification of MTSP3 as a serine protease required only 43% sequence identity. Similarly, Example 2 demonstrates that 37% sequence identity with MTSP1 was sufficient to identify MTSP4.

The Examples demonstrate additional features of the claimed polypeptides. For example, Examples 1, 2, 3 and 6 each demonstrate the expression of MTSP polypeptides in normal and tumor tissues. The working examples further demonstrate that each of the MTSP polypeptides, having, for example, 37-43% sequence identity, is active as a single chain protease domain.

The Examples demonstrate expression of single chain protease domains. For example, Example 1 describes the cloning of MTSP3 into an expression vector and expressing it in *E. coli*. The example describes the purification of the protein and the serine protease activity of the single chain protease domain using a variety of substrates. Examples 4 and 5 describe additional expression vector cloning techniques for *Pichia pastoris* expression for MTSP 3, 4 and 6. Example 5 provides a detailed example of a serine protease assay for the expressed MTSP6 single chain protease domain. Examples 6 and 7 provide a detailed description of the cloning, expression and purification of an MTSP1 single chain protease domain. Example 8 provides detailed serine protease assays for MTSP1. Additionally, Example 1 demonstrates that additional sequence variation can be introduced into single chain protease domains of an MTSP, such as a cysteine to serine change, without altering serine protease activity. Hence, the examples demonstrate the ability of one of skill in the art to isolate and express MTSP single chain polypeptides that include the protease domain without additional regions of MTSP sequence. The examples further demonstrates that one of skill in the art can identify MTSP sequences with 37-43% sequence identity that share common features of an MTSP and are active as single chain polypeptides.

As discussed above, the application provides the sequences and identities of at least a dozen MTSP family members and describes identification of the protease domain. One of skill in the art can readily isolate a protease domain as a single chain from any MTSP family member.

Predictability

The predictability at issue herein is whether one of skill in the art could isolate protease domains from MTSP family members and variants thereof, including serine protease family members that are single chain protease domains.

Applicant respectfully submits that one of skill in the art, given the instant disclosure, could predictably make such polypeptides, because the MTSP family is well known and the sequences of exemplary new family members, as well as known members, are provided in the application. One of skill in the art could readily make minor amino acid variation there, and, if needed, test such polypeptide variants for serine protease activity. Given the knowledge about the family members, the disclosure in the application, and the working examples, there is no doubt that isolation of protease domain from an MTSP is **reproducible. There is no doubt that one of skill in the art could prepare an isolated protease domain as claimed.**

In contrast to the allegations of "unpredictability" set forth in the Office Action, the specification and the knowledge in the art evidence many factors of *predictability* with respect to MTSP polypeptide variants. First, the specification provides more than a dozen exemplary polypeptides and identifies all family members, including the sequences thereof (in the sequence listing and/or by incorporation by reference of others) and also provides new family members. These are defined chemical structures from which one of skill in the art is given a reference point. As explained above, included among exemplary polypeptides are MTSP1, MTSP3, MTSP4-S, MTSP4-L and MTSP6.. The specification demonstrates that these MTSP polypeptides, as well as all family members, share conserved features including a protease domain with a catalytic triad and N-terminal activation cleavage site. Furthermore, the specification teaches isolation of the protease domains as single chains and demonstrates that they possess proteolytic activity. As discussed above, the specification provides detailed guidance for identifying a protease domain of any family member.

Second, the specification delineates structural and functional features of the protein. These features identify key regions and residues that one of skill in the art would know to conserve in order to retain serine protease activity. These features also provide reference points for alignments with other known serine proteases. These features also allow one of skill in the art to make further structure-function correlations, again providing predictable

correlations of regions and residues to conserve or change. As evidenced by the references cited in the specification and in the Information Disclosure Statements of record in this application, a large body of knowledge pertaining to structure-function relationships of serine proteases was known in the art. In addition, the specification provides exemplary assays to assess serine protease activity, including a variety of substrates for MTSP activity. . One of skill in the art could readily and routinely test any MTSP family member protease domain or a variant thereof for serine protease activity as a single chain protease.

As taught in the specification as well as evidenced by the art of record, maintenance of the catalytic triad is sufficient to retain serine protease activity. Therefore, one of skill in the art could make and generate MTSP family member protease domains from any MTSP known to one of skill in the art. In the unlikely event that it was needed, protease activity of could easily and routinely be confirmed using the assays provided in the application and known in the art. The routine manipulations to identify and isolate an MTSP protease domain as a single chain.

The experimentation necessary to make and use MTSP polypeptides, as described above, is routine. "Enablement is not precluded by the necessity for some experimentation such as routine screening. Experimentation needed to practice the invention must not be undue experimentation. 'The key word is *undue*, not experimentation.' " *In re Wands*, 858 F.2d at 737-38 (quoting *In re Angstadt*, 537 F.2d at 504; emphasis added; additional internal citations omitted). The Examiner admits that enzyme isolation techniques and recombinant and mutagenesis techniques are known and that it is routine to screen for multiple substitutions or multiple modifications as encompassed by the instant claims (see Office Action, page 11). The art related to serine proteases also demonstrates that such experimentation is not undue. For example, Pearson *et al.* ((1997) *Cabios Invited Review 13(4)*: 325-32) explains that serine proteases share a conserved catalytic site, the catalytic triad. In addition, trypsin-like serine proteases have several diagnostic motifs throughout the protein including a conserved protein fold and anti-parallel β barrel structures that contribute to the function of the protease. Pearson *et al.* states that one could recognize proteins that have protease activity based on these conserved structures. Hence, generation of variants with serine protease activity is routine because one of skill in the art can use such conserved features as a guide for designing the location of variations to maintain these features. In addition, Cheah *et al.* ((1990) *J. Biol. Chem. 265*:7180-7187) provides a demonstration of the predictability of generating variants of serine proteases based on an exemplary sequence. The authors use known structural and

functional information about trypsin-like serine proteases to obtain mutations in a rhinovirus 3C protease with predicted functional phenotypes. Thus, the art available at the time of filing, and before, demonstrates that one of skill in the art could make variants of a serine protease in a predictable manner. Therefore, one of skill in the art could make protease domains as single chains from an MTSP family member and also generate variants of MTSP polypeptides, -. Activity of the single chain protease domains and variants thereof could easily and routinely be confirmed using the assays provided in the application and known in the art. The routine manipulations to generate an MTSP single chain protease domains are not unpredictable.

As discussed above, the issue is not whether the claims encompass variant MTSPs, but whether one of skill in the art in possession of an MTSP could prepare an isolated protease domain in which a free Cys is replaced with a Ser.

The instant application identifies MTSP polypeptides that possess serine protease activity as a single chain. Such demonstration of single chain activity had not been demonstrated before the instant application. The application provides adequate description to demonstrate that a common feature among the MTSP family members is the activity of a single chain form that includes the protease domain or catalytically active portions thereof in the absence of other MTSP portions. The application provides exemplary MTSP's that share about 40% sequence identity and possess such features. Therefore, the specification demonstrates that by following the teachings of the application, one of skill in the art can predictably identify, make and use substantially purified polypeptides consisting of an MTSP protease domain or catalytically active fragment thereof having serine protease activity as a single chain.

The amount of experimentation required

There is nothing of record to suggest that production or use of any of the claimed polypeptides would require development of new procedures or excessive experimentation. Protein extraction, purification and synthesis methods have been used for decades. As discussed above, family members are provided in the application and well known in the art. In addition, if needed, assays for evaluating activity of the polypeptides are taught in the specification and are known in the art. Such assays are routine in this art and do not require excessive experimentation. The Examiner states that recombinant and mutagenesis techniques and enzyme isolation techniques are known and that it is routine to screen for multiple substitutions or multiple modifications as encompassed by the instant claims (see Office Action, page 11). Hence, the claimed polypeptides can be synthesized, isolated and characterized using routine testing, and, if necessary, one of skill in the art can test

polypeptides for catalytic activity by routine experimentation using the assays provided in the specification or known to those of skill in art. Applicant notes that "a considerable amount of experimentation is permissible, if it is merely routine . . ." *In re Wands* 858 F.3d 731, 737.

As discussed, mutagenesis methods are not required to make and use the polypeptides as claimed. The instant claims are directed to isolated protease domains of MTSP family members; one of skill in the art can identify the protease domain of any MTSP family member and find a free Cys and replace it as described in the application.

Conclusion

In light of the breadth of the claims, the extensive teachings and examples in the specification, the high level of skill of those in this art, the knowledge of those of skill in the art, and the fact that it is predictable to identify protease domains in MTSP family members and prepare single chain forms thereof as well as variants thereof, it would not require undue experimentation for one of skill in the art to make and use polypeptides with the features as claimed. Accordingly, a consideration of the factors enumerated above leads to the conclusion that undue experimentation would not be required to make and use the isolated MTSP protease domains as claimed.

V. REJECTION OF CLAIMS 1-3, 19 AND 20 UNDER 35 U.S.C. §102(a)

Claims 1-3, 19 and 20 are rejected under 35 U.S.C. § 102(a) as anticipated by Dawson *et al.* (US Pat. No. 5,645,833) because Dawson *et al.* allegedly discloses a polypeptide consisting of serine protease domain or a catalytically active fragment thereof of a MTSP protein. This rejection respectfully is traversed. It respectfully is submitted that claims 2, 3 and 19 are cancelled herein rendering this ground of rejection moot with respect to those claims.

Relevant Law

Anticipation requires the disclosure in a single prior art reference of each element of the claim under consideration. *In re Spada*, 15 USPQ2d 1655 (Fed. Cir, 1990), *In re Bond*, 15 USPQ 1566 (Fed. Cir. 1990), *Soundscriber Corp. v. U.S.*, 360 F.2d 954, 148 USPQ 298, 301, adopted 149 USPQ 640 (Ct. Cl.) 1966. See, also, *Richardson v. Suzuki Motor Co.*, 868 F.2d 1226, 1236, 9 USPQ2d 1913,1920 (Fed. Cir.), cert. denied, 110 S.Ct. 154 (1989). "[A]ll limitations in the claims must be found in the reference, since the claims measure the invention." *In re Lang*, 644 F.2d 856, 862, 209 USPQ 288, 293 (CCPA 1981). It is incumbent on Examiner to identify wherein each and every facet of the claimed invention is

disclosed in the reference. *Lindemann Maschinen-fabrik Gmbh v. American Hoist and Derrick Co.*, 730 F.2d 1452, 221 USPQ 481 (Fed. Cir. 1984).

The Claims

The claims are discussed above.

Disclosure of Dawson *et al.*

Dawson *et al.* discloses modified serine proteases of the chymotrypsin superfamily. The modifications to the serine proteases result in proteins that exhibit resistance to serine protease inhibitors. Figure 1 of Dawson *et al.* shows the alignment of the sequences of amino acids of the catalytic domain of the chymotrypsin superfamily.

Analysis

Claim 1 recites that the isolated substantially purified polypeptide consists of a protease domain or a smaller catalytically active portion of the protease as a single chain. Claim 1 also recites a free Cys residue of the serine protease domain of an MTSP polypeptide is replaced with another amino acid.

1) Dawson *et al.* does not disclose the expression, isolation or purification of any protease domain. Figure 1 in Dawson *et al.* merely sets for sequences. Figure 1 shows the alignment of the amino acid sequences of the catalytic domain of the chymotrypsin superfamily. The sequences shown in Figure 1 are a representation; they are not isolated polypeptides. Applicant respectfully submits that a comparison of sequences of protease domains is not a disclosure of an isolated, substantially purified single chain polypeptide consisting of an MTSP protease domain or smaller catalytically active portion thereof. Dawson *et al.* does not disclose an isolated polypeptide consisting of a catalytically active single chain protease domain of an MTSP polypeptide.

2) Dawson *et al.* does not disclose an isolated, substantially purified protease domain of an MTSP polypeptide having a free Cys residue that is replaced by another amino acid. Dawson *et al.* does not mention a free Cys residue, nor disclose replacement of any Cys residue.

Thus, Dawson *et al.* does not disclose every element of claim 1 nor any claims dependent thereon. Therefore Dawson *et al.* does not anticipate claim 1 or claim 20 nor any claim dependent thereon.

VI. REJECTION OF CLAIMS 1-3, 5, 11-13, 19, 20, 34-36, 40-42, 113 AND 114 UNDER 35 U.S.C. §102(a)

Claims 1-3, 5, 11-13, 19, 20, 34-36, 40-42, 113 and 114 are rejected under 35 U.S.C. § 102(a) as anticipated by Takeuchi *et al.* (Proc. Natl. Acad. Sci. USA 96: 11054-11061 (1999)) because Takeuchi *et al.* allegedly discloses a polypeptide comprising a serine protease domain that is 100% identical to amino acids 615-855 of SEQ ID NO:2, purifying a polypeptide comprising a fragment consisting of a serine protease domain that is identical to amino acids 615-855 of SEQ ID NO:2, that its polypeptide is not expressed on normal endothelial cells, is of human origin, consists essentially of the protease domain having catalytic activity and is expressed on tumor cells. The Examiner also alleges that the reference discloses a solid support that includes a polypeptide comprising a fragment consisting essentially of a serine protease domain that is identical to amino acids 615-855 of SEQ ID NO:2. This rejection respectfully is traversed. . It respectfully is submitted that claims 2, 3 and 19 are cancelled herein rendering this ground of rejection moot with respect to those claims.

Relevant Law

See related section above.

The Claims

The claims are discussed above.

Disclosure of Takeuchi *et al.*

Takeuchi *et al.* discloses a polypeptide that contains 855 amino acids and is designated MT-SP1. This protein has sequence identity with the full-length MTSP1 set forth as SEQ ID NO:2 of the instant application. Takeuchi *et al.* discloses an expression vector that includes nucleic acid encoding the protease domain ***plus*** the pro-domain (see page 11055, left col., third full paragraph). Takeuchi *et al.* discloses that its expression vector includes the mature protease domain and a small portion of the pro-domain and was designed to over-express the sequence encoding a polypeptide containing amino acids 596-855 with a His-tag fusion to produce as a construct Met-Arg-Gly-Ser-His_6-aa596-855 (page 11055, column 2, third full paragraph). Takeuchi *et al.* discloses that the pro-domain region is disulfide bonded to the protease domain of its construct (page 11055, column 2, third full paragraph). Takeuchi *et al.* discloses that amino acids Cys 604 and Cys 731 are disulfide bonded (see for example, at page 11060, col.1). Takeuchi *et al.* discloses that its protease domain is disulfide bonded to the pro-domain region (see page 11058, col. 1 and page 11060, col. 1, first paragraph) and remains bonded to the protease domain after activation (page 11058, lines 8-9).

Takeuchi *et al.* discloses that its "purified protease domain" includes the His-tag sequence, stating that a monoclonal antibody directed against the N-terminal Arg-Gly-Ser-His_4 epitope is immunoreactive with its purified protein (see page 11058). Figure 3 cited by the Examiner as showing an isolated protease domain is a *diagrammatic* representation of the MTSP1 protease domains; it by no means is an isolated protease domain. Furthermore, the figure depicts the disulfide bonds; and does not show a free Cys in the protease domain, nor a fragment consisting of the protease domain. Page 11057, referenced by the Examiner as describing isolation of protease domain, does not do so. The polypeptide is expressed as a His-tagged polypeptide which forms a two-chain structure by virtue of the Cys –Cys bonds depicted in Figure 3 and the His-tag extension. Furthermore, the paper discusses the activated His-tag extended polypeptide and describes its activity (see, *e.g.*, Figure 6 and page 11057, col. 2). Takeuchi states that:

> the MT-SP1 protease domain was expressed in *E. coli* as a His-tagged fusion and was purified from inclusion bodies under denaturing conditions by using metal-chelate affinity chromatography. . . . This denatured protein refolded when the urea was dialyzed from the protein. . . . N-terminal sequencing of the ***purified activated*** **[i.e. the two-chain folded form]** yielded the expected VVGGT activation sequence.

Thus, Takeuchi *et al.* expresses a His-tagged form of the protein that forms a two chain structure when activation cleaved. The sequenced molecules was the His-tagged protease domain. Takeuchi *et al.* does not disclose or contemplate an isolated polypeptide consisting of only the protease domain.

Further, it is apparent from the disclosure that Takeuchi believes that a two-chain structure is a requisite for activity. Takeuchi *et al.* discusses the need for activation cleavage and depicts the disulfide bond; there is no disclosure of a polypeptide in which there is a free Cys. Hence, there is no disclosure for replacing any Cys with a serine. There is no mention of replacement of any amino acids.

Hence Takeuchi *et al.* does not disclose isolation of a polypeptide consisting only of the protease domain of any MTSP, including an MTSP1. Its polypeptide includes a His-tag sequence; the active form of the enzyme includes a disulfide bond. In addition, the only isolation of a polypeptide comprising the protease domain (which includes the His-tag), was for sequencing purposes

Analysis

Claim 1 recites that the isolated substantially purified polypeptide consists only $$of a protease domain or a smaller catalytically active portion of the protease as a single chain, and

that a free Cys residue of the serine protease domain is replaced with another amino acid. Takeuchi *et al.* discloses that its protease domain is disulfide bonded to the pro-domain region and remains bonded to the protease domain after activation. Takeuchi *et al.* discloses that its "purified protease domain" includes the His-tag sequence, and states that a monoclonal antibody directed against the N-terminal Arg-Gly-Ser-His_4 epitope is immunoreactive with its purified protein. Thus, the "purified protease domain" disclosed by Takeuchi *et al.* includes additional amino acid residues in addition to the protease domain of the MT-SP1. Neither page 11057 nor Figure 3 disclose a single chain polypeptide that consists ***only*** of the protease domain. As discussed above, the protease domain as expressed and isolated by Takeuchi *et al.* includes additional amino acids. Takeuchi *et al.* states that:

> N-terminal sequencing of the ***purified activated*** **[i.e. the two-chain folded form]** yielded the expected VVGGT activation sequence.

The purified activated polypeptide according to Takeuchi *et al.* is a two chain polypeptide, and also, as expressed includes the His-tag for purification. Figure 3, as noted, is a diagrammatic representation of the full-length MTSP1 depicting the activated disulfide-bonded form of the enzyme (in which the Cys that is replaced in the instant claims, is part of the disulfide bond).

Hence, Takeuchi *et al.* does not disclose a polypeptide <u>consisting of</u> a protease domain or a smaller catalytically active portion of the protease domain. Takeuchi *et al.* discloses that its protease domain is disulfide bonded to the pro-domain region and remains bonded to the protease domain after activation and thus does not disclose a protease domain having a free Cys residue. Further, Takeuchi *et al.* does not disclose replacing a free Cys residue of the serine protease domain of an MTSP polypeptide with another amino acid. Thus, Takeuchi *et al.* does not disclose an isolated, substantially purified protease domain of an MTSP polypeptide having a free Cys residue replaced with another amino acid. Hence, the disclosure of Takeuchi *et al.* does not disclose every element of claim 1 and therefore does not anticipate the claimed subject matter. Therefore, Takeuchi *et al.* does not anticipate claim 1 nor any claim dependent thereon.

VII. THE REJECTION OF CLAIMS 1-3, 5, 10-14 AND 34 UNDER 35 U.S.C. §102(e)/103(a)

Claims 1-3, 5, 10-14 and 34 are rejected under 35 U.S.C. §102(e) as anticipated by O'Brien *et al.* or in the alternative obvious over O'Brien *et al.*, because it is alleged that the reference discloses a polypeptide with 100% identity to full-length MTSP1 as set forth in SEQ ID NO:2 of the instant application. It is further alleged that the polypeptide disclosed by O'Brien *et al.* inherently possess the features set forth in claims 2-3 and 6-9 of the instant

application. The Office Action also alleges that the reference discloses a protease domain identified therein as SEQ ID NO:14 that is 100% identical to amino acids 615-855 of SEQ ID NO:2. The Examiner states that O'Brien *et al.* does not disclose purifying the protease yet the Office Action concludes that the disclosed molecules in O'Brien *et al.* anticipate the claimed subject matter

In the alternative, it is alleged that the claims are obvious over the claimed subject matter because O'Brien *et al.* teaches a method of expressing polypeptides in host cells. It also is alleged that the reference teaches that the protease domain could be released from the polypeptide and used as a diagnostic that has the potential for therapeutic intervention. Thus, the Office Action concludes that it would have been obvious to one of skill in the art to express the protease domain disclosed as SEQ ID NO:14 by O'Brien *et al.* and purify the polypeptide. It is alleged that the motivation to make such polypeptides is the disclosed use as a diagnostic for therapeutic intervention. Further, it is alleged that one of ordinary skill in the art would have had a reasonable expectation of success since the expression of heterologous polypeptides was routine in the art and O'Brien *et al.* teaches how to express heterologous polypeptides. This rejection respectfully is traversed.

Relevant Law

The law with respect to anticipation under 35 U.S.C. § 102(a) is discussed above.

For *prima facie* obviousness of claimed subject matter to be established under 35 U.S.C. §103, all the claim limitations must be taught or suggested by the prior art. In re Royka, 490 F.2d 981, 180 USPQ 580 (CCPA 1974). This principle of U.S. law regarding obviousness was **not** altered by the recent Supreme Court holding in KSR International Co. v. Teleflex Inc., 127 S.Ct. 1727, 82 USPQ2d 1385 (2007). In KSR, the Supreme Court stated that "Section 103 forbids issuance of a patent when 'the differences between the subject matter sought to be patented and the prior art are such the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains.'" KSR Int'l Co. v. Teleflex Inc., 127 S.Ct. 1727, 1734, 82 USPQ2d 1385, 1391 (2007).

The mere fact that prior art may be modified to produce the claimed product does not make the modification obvious unless the prior art suggests the desirability of the modification. In re Fritch, 23 U.S.P.Q.2d 1780 (Fed. Cir. 1992); see, also, In re Papesh, 315 F.2d 381, 137 U.S.P.Q. 43 (CCPA 1963). Further, that which is within the capabilities of one skilled in the art is not synonymous with that which is obvious. *Ex parte Gerlach*, 212 USPQ 471 (Bd. APP. 1980).

Furthermore, the Supreme Court in KSR took the opportunity to reiterate a second long-standing principle of U.S. law: that a holding of obviousness requires the fact finder (here, the Examiner), to make explicit the analysis supporting a rejection under 35 U.S.C. 103, stating that "rejections on obviousness cannot be sustained by mere conclusory statements; instead, there must be some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness. Id. at 1740-41, 82 USPQ2d at 1396 (citing In re Kahn, 441 F.3d 977, 988, 78 USPQ2d 1329, 1336 (Fed. Cir. 2006)).

While the Supreme Court in KSR rejected a rigid application of the teaching, suggestion, or motivation ("TSM") test in an obviousness inquiry, the Court acknowledged the importance of identifying "a reason that would have prompted a person of ordinary skill in the relevant field to combine the elements in the way the claimed new invention does" in an obviousness determination. KSR, 127 S. Ct. at 1731. Moreover, the Court indicated that there is "no necessary inconsistency between the idea underlying the TSM test and the Graham analysis." *Id.* As long as the test is not applied as a "rigid and mandatory" formula, that test can provide "helpful insight" to an obviousness inquiry. *Id.* Thus, in cases involving new chemical compounds, it remains necessary to identify some reason that would have led a chemist to modify a known compound in a particular manner to establish *prima facie* obviousness of a new claimed compound." Takeda Chem. Indus., Ltd. v. Alphapharm Pty., Ltd. (No. 2006-1329 (Fed. Cir. 2007)).

The Claims

Claims 1, 5,10-13 and 34 are discussed above.

The disclosure of O'Brien *et al.*

O'Brien *et al.* discloses a protein identified therein as TADG-15, which is an MTSP1 variant, with a sequence of amino acids as set forth as SEQ ID NO:2. The reference also discloses a comparison of the amino acid sequence of the protease domain of TADG-15 (SEQ ID NO:14) with other serine protease catalytic domains (see Figure 2). O'Brien *et al.* discloses that TADG-15 is a highly over-expressed gene in tumors and suggests that TADG-15 is novel in its component structure of domains because it has a protease catalytic domain that *could* be released and used as a diagnostic and that *potentially could be* a target for therapeutic intervention (col. 15, lines 31-38):

> TADG-15 is a highly overexpressed gene in tumors. It is expressed in a limited number of normal tissues, primarily tissues that are involved in either uptake or secretion of molecules e.g. colon and pancreas. TADG-15 is further novel in its component structure of domains in that it has a protease catalytic

domain which could be released and used as a diagnostic and which has the potential for a target for therapeutic intervention

Thus, O'Brien states that the TADG-15 protease domain possibly could be released *in vivo* and serve as a **therapeutic target**, not as a therapeutic O'Brien *et al.* does not disclose, teach or suggest or mention or even hint at isolating the protease domain.

O'Brien *et al.* does not disclose, teach or suggest isolation of the protease domain as a single-chain polypeptide that ***consists only of the protease domain as a single chain.*** O'Brien *et al.* does not disclose, teach or suggest a protease domain of an MTSP polypeptide that has a free Cys residue, or replacing a free Cys residue of a serine protease domain of an MTSP polypeptide with another amino acid.

A. THE ANTICIPATION REJECTION

Claim 1 recites that the isolated substantially purified polypeptide consists of a protease domain or a smaller catalytically active portion of the protease as a single chain, and that a free Cys residue of the serine protease domain is replaced with another amino acid. O'Brien *et al.* does not disclose an isolated polypeptide that consists of a protease domain or a smaller catalytically active portion of the protease as a single chain. O'Brien *et al.* does not disclose an isolated single-chain protease domain of an MTSP polypeptide having a free Cys residue, or replacing a free Cys residue of an isolated single-chain serine protease domain of an MTSP polypeptide with another amino acid. The Examiner acknowledges that O'Brien *et al.* does not disclose a protease domain of an MTSP polypeptide where a free Cys residue in the protease domain is replaced with Ser residues (see Office Action at page 25). In addition, as discussed, O'Brien *et al.* does not disclose an isolated protease domain of an MTSP. Stating that such protease domain could be release *in vivo* and used as a diagnostic target does not constitute a disclosure of an isolated single chain protease domain, and certainly does not constitute disclosure of an isolated protease domain in which a free Cys is replaced.

Hence, O'Brien *et al.* does not disclose every element of claim 1 as claimed. Therefore O'Brien *et al.* does not claim 1 nor any claim dependent thereon.

B. THE OBVIOUSNESS REJECTION

Claims 1-3, 5, 10-13 and 34 as well as claims 35, 36, 40-42, 113 and 114, are rejected unpatenable over O'Brien *et al.* because because O'Brien et al. teaches a method of expressing polypeptides in host cells and that it teaches that that the the protease domain could be released from the polypeptide and used as a diagnostic that has the potential for therapeutic intervention. This rejection is respectfully traversed.

This rejection is respectfully traversed.

As discussed above, O'Brien *et al.* states that:

> TADG-15 is a highly overexpressed gene in tumors. It is expressed in a limited number of normal tissues, primarily tissues that are involved in either uptake or secretion of molecules e.g. colon and pancreas. TADG-15 is further novel in its component structure of domains in that it has a protease catalytic domain which could be released and used as a diagnostic and which has the potential for a target for therapeutic intervention

O'Brien *et al.* is speculating that the protease domain could be released *in vivo* and serve as a ***therapeutic target***. O'Brien *et al.* does not teach or suggest that the protease domain exists even *in vivo* as a single chain, and does not teach or suggest isolating it. In this passage, noted by the Examiner, O'Brien *et al.* is discussing the expression of TADG-15 in tumors and other tissues and indicates that it is expressed on the surface of cells. Because of its stucture, the protease domain could be presented on the surface of cells *in vivo*, and, thus, "could be released." Since it is over expressed in tumors, if released *in vivo*, it could serve as a diagnostic marker indicating the presence of tumor cells. Use of its presence *in vivo* as a diagnostic marker for detection of tumors and/or as a therapeutic target is not a teaching or suggestion or hint for isolating the protease domain, nor for producing it as a single-chain polypeptide, nor for modifying it by replacing what would be a free Cys in a single chain form with another amino acid.

Thus, O'Brien *et al.* does not state or hint that the *isolated* single chain protease domain could be used as therapeutic or as a diagnostic, and certainly does not teach or suggest then modifying it by replacing what is free Cys in the single chain polypeptide. Such teaching does not constitute even a hint or suggestion for isolation or productin of a polyeptide consisting only of the single-chain protease domain of an MTSP, nor of a single chain protease domain in which the free Cys (which results only by virtue of it being a single chain) is replaced with another amino acid.

Therefore, the Examiner has failed to set forth a case of *prima facie* obviousness.

O'Brien *et al.* does not teach or suggest an isolated single chain protease domain of an MTSP polypeptide nor one in which a free Cys residue is replaced with another amino acid, such as a serine. There is no teaching or suggestion in O'Brien *et al.* for preparing a polyeptide consisting only of a single-chain protease domain and modifying by replacing what is a free Cys in the single-chain form with another amino acid. Even post-KSR, "it remains necessary to identify some reason that would have led a chemist to modify a known

compound in a particular manner to establish prima facie obviousness of a new claimed compound." *Takeda Chem. Indus., Ltd. v. Alphapharm Pty., Ltd.* (Fed. Cir. 2007).

In this instance, there is not teaching or suggestion in O'Brien *et al.* for isolating a single chain polypeptide consisting only of an MTSP protease domain in which a free Cys is replaced. O'Brien *et al.* provides no teaching or suggestion for isolating the protease domaina and preparing it as a single chain, and none for modifying the resulting single-chain polypeptide. Thus, the Examiner has failed to set forth a *prima facie* case of obviousness of claim 1 and, thus, claims 5, 10-13, 34-36, 40-42, 113 and 114, which ultimately depend from claim 1.

VIII. REJECTION OF CLAIMS 19 AND 20 UNDER 35 U.S.C. §103(a)

Claims 19 and 20 are rejected under 35 U.S.C. § 103(a) as being unpatentable over O'Brien *et al.* (U.S. Patent No. 5,972,616) and Estell *et al.* in view of Takeuchi *et al.* because it O'Brien *et al.* teaches a serine protease domain of an MTSP polypeptide but does not teach replacing free Cys residues with Ser residues, but Estell *et al.* in light of Takeuchi *et al.* allegedly cures this defect. The Examiner alleges that it was well known in the art that proteins form disulfide bonds through SH groups of Cys residues. It is alleged that Takeuchi *et al.* teaches that position 731 normally forms a disulfide bond with a Cys residue in the pro-protease domain. The Office Action alleges that Estell *et al.* teaches that Cys residues replaced with Ser residues decrease a polypeptide's susceptibility to oxidation. The Office Action concludes that it would have been obvious to one of ordinary skill in the art to replace a free Cys residue in the protease domain taught by O'Brien *et al.* with a Ser residue in order to enhance stability of the protein. It is alleged that there would have been a reasonable expectation of success because Estell *et al.* teaches that such changes successfully decrease a protein's susceptibility to oxidation. This rejection respectfully is traversed.

Relevant Law

See related section above.

The Claims

Claim 19 is cancelled herein, rendering any rejection with respect to claim 19 moot. Claim 20 depends from claim 1 and recites that the free Cys is replaced with a serine.

Teachings of the Cited References

O'Brien *et al.* and Takeuchi *et al.*

The teachings of O'Brien *et al.* and Takeuchi *et al.* are discussed above. As discussed above, neither reference teaches or suggests isolating a single chain polypeptide that consists only

of the protease domain of an MTSP. Further, Takeuchi *et al.* unequivocally teaches that the protease domain must fold and form disulfide bonds for activity.

Estell *et al.*

Estell *et al.* teaches a method for producing prokaryotic carbonyl hydrolase enzymes, including subtilisin, in recombinant host cells. Estell *et al.* teaches methods for making a mutant Bacillus subtilisin having altered oxidative stability by:

> obtaining DNA fragment consisting of a region coding for a Bacillus subtilisin, and introducing a mutation into said DNA fragment such that the mutation is introduced in a region encoding a methionine, tryptophan, cysteine or lysine, sensitive to oxidation, such that upon expression of the mutant subtilisin one or more codon regions encoding for methionine, tryptophan, cysteine or lysine is replaced with an amino acid other than methionine, tryptophan, cysteine or lysine, preferably alanine or serine.

The method of Estell *et al.* includes introducing mutations into a region encoding a methionine, tryptophan, cysteine or lysine to render the enzyme less sensitive to oxidation by reaction of such residue. Takeuchi *et al.*, however, teaches that the Cys in the protease domain forms disulfide bonds in the activated form. Hence replacement of such Cys would prevent formation of an activated form of the enzyme. There is no suggestion in Takeuchi *et al.*,, or in Estell *et al.* nor any reference of record, that the Cys, that in a single chain form of a protease domain is 1) free and 2) susceptible to oxidation. Hence there is no suggestion in Estell nor any reference of record, that the Cys, that in a single chain form of a protease domain is 1) free and 2) susceptible to oxidation. Hence there is no suggestion in Estell *et al.*, or Takeuchi *et al.* or O'Brien *et al.* to replace such Cys with a another amino acid and thereby replace a residue that is necessary for activity.

A teaching that one can replace methionine, tryptophan, cysteine or lysine in bacterial subtilisin is not a teaching or suggestion for **replacing a *free* Cys that exists only in the protease domain of an MTSP when it is in the single chain form**. None of the references teaches or suggest preparing the protease domain as a single chain form, and certainly none suggest then replacing the Cys needed to produce the activated form.

Analysis

The Examiner has failed to set forth a case of *prima facie* obviousness because the combination of teachings of the references does not result in the instantly claimed polypeptides

Claim 20 ultimately depends from claim 1 and includes every limitation thereof. As discussed above, neither O'Brien *et al.* nor Takeuchi *et al.* teaches or suggests every limitation

of claim 1. Neither Estell *et al.* nor Estell *et al.* and Takeuchi *et al.* does not cure the deficiencies in the teachings of O'Brien *et al.* and/or Takeuchi *et al.*

Neither O'Brien *et al.* nor Takeuchi *et al.* teaches or suggests preparing a **single chain** polypeptide that consists only of the protease domain of an MTSP. Further, Takeuchi *et al.* clearly teaches states throughout that the Cys in the protease domain forms disulfide bonds needed for activation of the protease domain. There can be no teaching or suggestion for replacement of the Cys that would be free only in the single-chain form of the protease. Estell *et al.* does not cure this deficiency.

As noted, there is no teaching or suggestion by Takeuchi *et al.* to generate a single-chain polypeptide or that the protease domain as a single-chain without its disulfide bonded pro-domain portion would be catalytically active. Further, Takeuchi *et al.* does not teach or suggest an MTSP protease domain having a free Cys residue. As the Examiner points out, Takeuchi *et al.* teaches that the cysteine residue at position 731 of SEQ ID NO:2 forms a disulfide bond with a cysteine residue in the pro-domain (see page 11060 top left paragraph and Figure 4). Takeuchi *et al.* teaches that its protease domain is disulfide bonded to the pro-domain region (see page 11058, col. 1 and page 11060, col. 1, first paragraph) and remains bonded to the protease domain after activation (page 11058, lines 8-9). Takeuchi *et al.* teaches that its "purified protease domain" includes the His-tag sequence, stating that a monoclonal antibody directed against the N-terminal Arg-Gly-Ser-His_4 epitope is immunoreactive with its purified protein (see page 11058). Thus, Takeuchi *et al.* teaches that the Cys residue in the protease domain is disulfide-bonded at least to the His-tag sequence. Hence, the "purified protease domain" of Takeuchi *et al.* does not include a free Cys residue. Thus, Takeuchi *et al.* does not teach or suggest an MTSP protease domain having a free Cys residue.

Estell *et al.* fails to remedy the deficiencies in the teachings of O'Brien *et al.* and Takeuchi *et al.*, singly or in combination. Estell *et al.* does not teach or suggest any single-chain polypeptides that consist of an MTSP protease domain or catalytically active portion thereof. Estell *et al.* does not teach or suggest that an isolated MTSP protease domain or catalytically active portion thereof has activity as a single-chain polypeptide. Estell *et al.* does not teach or suggest an MTSP protease domain having a free Cys residue. Estell *et al.* teaches that in Bacterial subtilisin a methionine, tryptophan, cysteine or lysine can be replaced to increase oxidative stability. Estell *et al.* does not teach or suggest replacing any and all such residues nor replacing residues that are thought to participate in forming an activated enzyme

Thus, O'Brien *et al.*, Takeuchi *et al.* and Estell *et al.*, alone or in any combination, does not teach or suggest an isolated single-chain MTSP protease domain, nor replacement of a free Cys in such single chain polypeptide with another amino acid. Therefore, the Examiner has failed to set forth a *prima facie* case of obviousness of any pending claim.

Comment regarding that examiner's arguments:

The Examiner again alleges that the applicant in the previous response argued that that the combination of the teachings of O'Brien *et al.* with the teachings of Estell *et al.* and Takeuchi *et al.* does not result in the instantly claimed methods. Applicant respectfully submits that no arguments directed to methods were made in the previous response. In the previous response, Applicant argued, in part, that:

> None of O'Brien *et al.*, Takeuchi *et al.* and Estell *et al.*, alone or in any combination, teaches or suggests polypeptides with the features set forth in claim 1: a single chain polypeptide that includes an MTSP protease domain or smaller portion thereof as the only MTSP portion of the polypeptide where the protease domain or smaller portion thereof has catalytic activity as a single chain. In view of the failure of the references, alone or in any combination, to teach or suggest the polypeptides of claim 1, the combination of the references does not teach or suggest the polypeptides of dependent claims 16, 18-20 and 137, which include all of the limitations of claim 1. Thus, the combination of the teachings of O'Brien *et al.*, Takeuchi *et al.* and Estell *et al.* does not render any of the claimed subject matter obvious. Therefore, the Examiner has failed to set forth a *prima facie* case of obviousness.

Hence, no argument was made in the previous response directed to methods.

* * *

In view of the amendments and remarks herein, reconsideration and allowance of the application are respectfully requested.

Respectfully submitted,

Stephanie Seidman
Reg. No. 33,779

Attorney Docket No. 17106-017001/1607
Address all correspondence to: 20985
Stephanie Seidman, Ph.D.
Fish & Richardson P.C.
12390 El Camino Real
San Diego, California 92130
Telephone: (858) 678-5070
Facsimile: (202) 626-7796
email: seidman@fr.com